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BIOCHEMICAL TESTS FOR THE DIFFERENTIATION OF KLEBSIELLA AND CLOACA

E. Hormaeche[1] and M. Munilla

The latest report (1) of the Enterobacteriaceae Subcommittee (1953) defines the Klebsiella group, (including Aerobacter aerogenes) as made up of nonmotile, indole-negative, MR-negative, VP-positive, citrate-positive, non-gelatin-liquefying, Gram-negative rods. They also decompose urea, although their urease activity is much weaker than that of Proteus. While all these biochemical characters are considered typical of the group, the report also mentions that included in the group are individual cultures which give aberrant results in one or more of these tests. Although it was realized that motile and nonmotile strains had many characters in common, as the Subcommittee eliminated motile strains from Klebsiella, it was possible to correlate motility with biochemical behaviour. There are, therefore, sound reasons for placing these motile strains in a special group of their own, the Cloaca group.

Brooke (2) compared 108 cultures of Cloaca, 71 of them motile, with 303 nonmotile Klebsiella strains. He found that all strains of Cloaca liquefied gelatin, none produced gas from glycerol, one fermented inositol, one dulcitol, 20 were urea-positive and only 9 promptly fermented salicin. All 303 Klebsiella cultures produced gas from glycerol, 296 promptly fermented inositol, 91 dulcitol, 17 liquefied gelatin, 300 were urea-positive and 302 fermented salicin in 24 hours. He concluded that gelatin liquefaction, motility, inability to produce gas from glycerol, and to ferment inositol, are the best characters to identify Cloaca, while dulcitol, salicin and urea tests may also be useful. His cultures of Klebsiella included 13 MR-positive strains, 12 indole producers, and 1 VP-negative strain. Cellobiose and starch were not used by Brooke. For the urea test he used the Christensen medium as modified by Kristensen, with phenol red indicator as recommended by Kauffmann (3). Moeller (4) in his excellent paper

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on the biochemical behaviour of the Enterobacteriaceae included 89 strains of Klebsiella, one of them motile, and 27 cultures of Cloaca, 2 of them "O" forms. Both groups fermented cellobiose and most of them were positive in the KCN test. As distinguishing characters he found that 100 per cent of the klebsiellae fermented inositol, but in contrast with Brooke's results, so did 26 per cent of the strains of Cloaca; neither glycerol nor starch was used. Adonitol was fermented by 94 per cent of Moeller's Klebsiella and 26 per cent of Cloaca strains. Gelatin was liquefied by 11 per cent of Klebsiella strains, but 11 per cent of Cloaca were negative. With salicin his figures are 99 per cent positive for Klebsiella and 100 per cent for Cloaca in three days, and with dulcitol 26 per cent and 11 per cent respectively. For the urea test he used the same medium as Brooke and found 87 per cent positive in Klebsiella and 63 per cent in Cloaca. Therefore it appears that there is no strict correlation between motility and any of these tests, gelatin liquefaction remaining as the best single test for differentiation of the two groups.

In a later paper Moeller (5) mentions the results of his improved and simplified tests for decarboxylases, 86 Klebsiella and 27 Cloaca strains were tested. The first were all negative in the arginine test and 2 per cent positive with ornithine; cultures of Cloaca were respectively 93 per cent and 96 per cent positive. All gave negative tests with glutamic acid, and with lysine all strains of Klebsiella were positive while those of Cloaca were 52 per cent positive, including delayed and weak reactions. Apparently thus far, arginine and ornithine tests are best for distinguishing the two groups.

Edwards and Fife (6) tested 128 motile and 626 nonmotile cultures. Of the motile, 61 per cent fermented inositol, 51 per cent adonitol, 58 per cent produced gas from glycerol (including delayed reactions), 28 per cent were urea-positive, 21 per cent fermented dulcitol and 99.3 per cent salicin; the positive tests from the second group were respectively 98, 92, 92, 92, 30 and 99.6 per cent. With soluble starch 1.9 per cent of motile cultures produced gas in four days, while 61 per cent fermented it without gas, against 99 per cent nonmotile fermenting it and 82 per cent producing gas. Gelatin was liquefied by 99 motile and by 28 nonmotile strains. Cellobiose and KCN tests were not used. The authors arrive at the conclusion that no single biochemical test or combination of tests is clearly sufficient to distinguish Klebsiella from Cloaca, and that many aberrant and intermediate strains occur.

They consider motility and gelatin liquefaction to be the best distinguishing characters, but they think that among their nonmotile strains there may be some "O" forms of Cloaca, which seems probable on the basis of their results.

At present the situation is confusing since there exist such discrepancies concerning the value of all known criteria, and Moeller's decarboxylase tests have not yet, to our knowledge, been confirmed by others. The classification of many strains may present insurmountable difficulties and there is even a certain reluctance to admit as Klebsiella some of the serotypes considered as aberrant, either because of their IMViC reactions or because they liquefy gelatin. While admittedly the problem is far from solved, it is our purpose to show in this paper which tests we believe to be the best. By their use it is possible and even easy for us to classify by their biochemical behaviour most strains with which we have to deal, although there remain some "intermediates" as is to be expected. We would like to add that in our opinion distinguishing between Klebsiella and Cloaca is not as difficult as between them and the related groups, Escherichia coli, E. freundii, and Serratia, a subject which we intend to discuss in a later paper.

MATERIAL

The 72 Klebsiella cultures representing the accepted serological capsular types mentioned in Table 1 were obtained from Drs. F. Kauffmann and P. R. Edwards, to whom we express our gratitude. All strains in Table 2 were isolated by us. Dr. Edwards sent us at our request many motile and nonmotile strains which are not included in our tables. With the exception of 43 motile cultures from Dr. Edwards, all the remaining strains in Table 3 were isolated in Montevideo, most of them from human stools, many from other human materials and many also from cow's milk and from water. Not included in our tables are 2 nonmotile and 6 motile anaerogenic strains, which we have left out as they would oblige us to tabulate them separately. They are of no importance in this study as they behaved like the aerogenic types. Fifteen more anaerogenic, motile cultures resembling Cloaca were classified as Serratia on the basis of their behaviour with respect to gelatin liquefaction, and milk peptonization, and to cellobiose, rhamnose and arginine tests. We would also like to thank

Dr. Cowan, curator of the National Collection of Type Cultures, for sending us many cultures used as reference and Dr. V. Moeller for testing about 40 of our cultures for decarboxylases.

METHODS

Motility was determined both by direct observation in hanging drops of young broth cultures, and by the use of semi-solid medium as recommended by Edwards and Brunner. Nonmotile forms showing the biochemical characters of Cloaca were kept in the semisolid medium for months both at 37° C. and at room temperature with periodic transfers. In this way we obtained motile cultures on several occasions. For capsule observation we employed the Burri method as recommended by the author for Spirochaeta with Gunther Wagner "Pellikan 541" India ink.

The fermentation of carbohydrates was tested in broth prepared with beef extract, using the local product from the Frigorifico Nacional 0.5 per cent, Evans' peptone 1.0 per cent, and NaCl 0.5 per cent. The pH was adjusted to 7.2-7.4 and phenol red used as indicator. With the exception of lactose, glucose, starch and glycerol, which were used at 1 per cent, all carbohydrates were added in the proportion of 0.5 per cent, Seitz filtered, and all tubes were fitted with gas bells, as it is our experience that to observe gas production from glucose only in misleading. Some strains anaerogenic in glucose produce gas from a different carbohydrate; while transfers made from tubes showing gas are frequently gas positive in glucose, this is not always the case. Tubes were cork stoppered and kept under observation for one month, although we do not think it necessary to observe them longer than 15 days, save in the case of milk and gelatin. Gas production from glycerol was read after four days.

For starch we followed the recommendation of Levine (7) who, as far back as 1918 used nonsoluble starch and rightly advocated its usefulness for the differentiation of Klebsiella and Cloaca. To avoid lumps and assure uniformity of the medium the starch is finely ground, added to cold water, the mixture stirred and boiled 5 minutes. The resulting jelly is added to the basic medium, distributed in sterilized tubes fitted with gas bells, and sterilized at 110° C. for 15 minutes. The tubes were inoculated with a loopful of 24-hour broth

culture, observed daily, and if on the fourth day there was no trace of gas in the bell, a red-hot wire loop was inserted into the medium. In this way we had some positive reactions when no gas was visible, and in doubtful cases we found it convenient to make transfers to new tubes where gas production was generally much quicker. Soluble starch is not so satisfactory as it is mostly dextrin. The results with dextrin are tabulated with the other tests.

The aesculin test was also performed according to Levine, with slight modifications. Bacto-peptone 0.5 per cent, and K_2HPO_4 0.1 per cent, was used as the base. After sterilization, aesculin 0.3 per cent and iron citrate 0.05 per cent, Seitz filtered, were added, and the medium distributed in small tubes without gas bells. Positive reactions were recognized by an intense black colour, while no change or different shades of brown were considered as negative. Readings must take place after no more than 24 hours of incubation, to obtain the best differentiation.

For the MR and VP reactions the medium as recommended by Kauffmann (3) was employed, with the exception that we used Witte instead of Orthana peptone. For the VP test the Barritt method was used, readings being taken after 2 and 4 days. MR was tested after 4 days at 37° C. For citrate utilization we used the Koser formula. Litmus skimmed milk was employed for the tests of coagulation and peptonization, nitrate reduction was tested in the medium recommended by Kauffmann (3). Difco gelatin was used for liquefaction, incubated at 37° C., cooled to 20° C. for reading and observed for one month. For H_2S production we used a medium composed of beef extract 0.75 per cent, Bacto-peptone 2 per cent, NaCl 0.5 per cent and sodium thiosulfate 0.008 per cent, pH 7.4-7.6. Strips of filter paper impregnated in a saturated solution of lead acetate were inserted between the tubes and the stoppers. Readings were made after 2 days incubation. For indole we employed a solution of Bacto-tryptone 1 per cent, NaCl 0.5 per cent with a pH of 7-7.2; strips of paper impregnated in oxalic acid solution were used to detect indole and when negative, Ehrlich reagent was added to the medium after 48 hours incubation. For the KCN test we used Moeller's formula with Evans' peptone 0.6 per cent instead of Orthana which was not available.

The urea test was carried out in two different ways: 1) the "slow" test, using the Christensen medium modified by Kristenson as recommended by Kauffmann but using as indicator 24 ml. per liter of a 0.04 per cent cresol red instead of

phenol red. This change in the indicator proved to be very satisfactory as cresol red allows the detection of a higher pH and eliminates positive reactions with Cloaca; and 2) the "quick" test which is performed as follows: a 2 per cent solution of urea is prepared, 2 ml. of cresol red solution as above are added, and distributed in small (hemolysis) tubes containing 1 ml. each. No sterilization is needed. The tubes are inoculated with two heavy loopfuls of a 24-hour agar culture and incubated at 45-50° C. for two hours, when positive reactions are recognized by violet-red colour. Cultures older than 24 hours are generally too alkaline and are not suitable. The best results are obtained with growth from dry agar plates. Montevideo tap water is excellent to prepare the urea solution for the test, but when distilled water is used it is convenient to add monopotasium phosphate 1/10,000 to eliminate confusing weak reactions produced by some Cloaca cultures. It is also recommended not to use bacteria grown on lactose agar, as under those conditions most Cloaca cultures will give a positive reaction. The arginine test was performed using the medium as recommended by Moeller but without the addition of the decarboxylase co-enzyme pyridoxal. In this way the reaction that takes place is apparently not due to decarboxylase but to the dihydrolase system as proved by the fact that without exception positive tubes gave a strong positive NH_3 test. This arginine test was found useful not only to differentiate Cloaca from Klebsiella and Serratia, as shown by Moeller, but it has also the advantage that most Escherichia cultures are negative. While Moeller found that 99 per cent of his "coli" strains were positive with his decarboxylase method, we had only 13 positive in a series of 80 such cultures. For the preparation of the medium we used the local beef extract and Evans' peptone instead of Orthana brand recommended by Moeller. We have not enough experience with lysine, ornithine and glutamic acid decarboxylases to arrive at any conclusions.

RESULTS

The following tables summarize the most important characters which differentiate Klebsiella from Cloaca. As gas production from some carbohydrates is one of them, in order to avoid confusion we have tabulated anaerogenic cultures separately. We have grouped all of them according to their

Table 1. The 72 Klebsiella capsulated strains from type culture collections.

	Gas from cellobiose								Acid from cellobiose		
Group	1	2	3	4	5	6	7	8	1	3	4
IMViC											
No. of strains	53	5	1	1	1	4	1	3	1	1	1
Dulcitol	Gas:17 Neg:36	Gas:1 Neg:4	Neg.	Neg.	Neg.	Gas:1 Neg:2 Ac: 1	Neg.	Gas:2 Neg:1	Neg.	Neg.	Neg.
Adonitol	Neg: 8 Gas:45	Gas:5	Gas	Gas	Gas	Gas:4	Gas	Gas:3	Acid	Acid	Acid
Inositol	Ac: 1 Gas:52	Gas:5	Gas	Gas	Gas	Gas:4	Gas	Gas:3	Acid	Acid	Acid
Aesculin	Neg: 1 Pos:52	Neg:1 Pos:4	Pos.	Pos.	Pos.	Neg:1 Pos:3	Pos.	Pos:3	Neg.	Pos.	Pos.
Starch	Gas:53	Gas:5	Gas	Neg.	Gas	Gas:4	Gas	Gas:3	Acid	Acid	Acid
Dextrin	Ac: 2 Gas:51	Gas:5	Gas	Acid	Gas	Ac: 1 Gas:3	Gas	Gas:3	Acid	Acid	Acid
Glycerol	Gas:53	Gas:5	Gas	Gas	Gas	Gas:4	Gas	Gas:3	Acid	Acid	Acid
Urea (slow)	Pos:49 Neg: 4	Pos:2 Neg:3	Neg.	Neg.	Pos.	Pos:4	Pos.	Pos:3	Pos.	Neg.	Pos.
Urea (quick)	Pos:50 Neg: 3	Pos:2 Neg:3	Neg.	Neg.	Pos.	Pos:4	Pos.	Pos:3	Pos.	Neg.	Neg.
Gelatine	Pos: 3 Neg:50	Neg:5	Pos.	Neg.	Pos.	Pos:3 Neg:1	Neg.	Neg:3	Neg.	Neg.	Neg.
KCN	Pos:52 Neg: 1	Pos:5	Pos.	Pos.	Pos.	Pos:4	Pos.	Pos:3	Pos.	Weak Pos.	Weak Pos.
Arginine	Neg:53	Neg:5	Neg.	Pos.	Neg.	Neg:4	Neg.	Neg:3	Neg.	Neg.	Neg.

IMViC reactions and it will be seen that there were 9 different combinations among Klebsiella and 5 among Cloaca. We are not suggesting that these different combinations represent subdivisions in either group, our purpose is only to show that whatever the IMViC reactions may be, the remaining properties have enough in common to place all these cultures in one group or another, and that there is a close correlation between motility and biochemical behaviour.

All the Klebsiella serotype strains from Kauffmann and Edwards fermented (with gas the aerogenic, and without it the anaerogenic), glucose, saccharose, mannitol, maltose, salicin, sorbitol, arabinose and xylose. With the exception of one culture of group 1, which produced acid only, all fermented rhamnose with gas. Four did not coagulate milk, none peptonized it, none produced H_2S, two did not produce nitrites from nitrates. Lactose fermentation was delayed with serotypes 1, 3, 4, 5 and 6, while all the rest promptly fermented it with abundant gas.

All locally isolated strains of Klebsiella fermented (with gas production) lactose, glucose, saccharose, mannitol, maltose, salicin, sorbitol, arabinose and xylose. One did not ferment rhamnose, another did not produce gas from it. Three did not coagulate milk, none peptonized it. All reduced nitrates to nitrites, none produced H_2S, all were nonmotile.

All strains of Cloaca fermented glucose, mannitol, maltose, salicin and arabinose with production of gas; 17 fermented lactose without producing gas, 1 did not ferment it, 1 did not ferment saccharose, 9 did not ferment rhamnose, 1 fermented rhamnose but without gas; 8 did not ferment sorbitol; all save one produced gas from xylose. Two cultures did not coagulate milk, none peptonized it; 2 did not reduce nitrates to nitrites. No H_2S production was observed in any case.

Tables 1 and 2 combining the 69 aerogenic Klebsiella serotype strains and our own 286 strains, 355 in total, gave 351 which produced gas in 4 days from nonsoluble starch, or 98.9 per cent as contrasted with 4 that did not. Compared with these only one of our 177 motile cultures of Cloaca was positive, or 0.56 per cent, and even this strain, which we got from Dr. Edwards and belongs to the glycerol-and-inositol-positive, arginine-negative group mentioned below, behaved in a peculiar way. At first it produced just a small bubble of gas and only after several transfers in the same medium was there enough gas to fill the glass bell. It was then

Table 2. The 286 *Klebsiella* isolated strains. All produce gas from cellobiose.

Group	1	2	4	5	6	7	8	9
IMViC								
No. of strains	198	16	14	12	32	2	11	1
Dulcitol	Ac: 1 Gas:67 Neg:130	Gas:2 Neg:14	Gas:6 Neg:8	Gas:4 Neg:8	Gas:16 Neg:16	Neg:2	Gas:4 Neg:7	Gas
Adonitol	Ac: 1 Gas:157 Neg:40	Gas:14 Neg: 2	Gas:13 Neg: 1	Gas:12	Gas:32	Gas:2	Gas:11	Gas
Inositol	Ac: 4 Gas:193 Neg:1	Gas:16	Gas:13 Neg:1	Gas:12	Gas:32	Gas:2	Gas:10 Ac: 1	Gas
Aesculin	Pos:197 Neg:1	Pos:16	Pos:14	Pos:12	Pos:32	Pos:2	Pos:11	Pos.
Starch	Ac: 2 Gas:196	Ac: 1 Gas:15	Gas:14	Gas:12	Gas:32	Gas:2	Gas:11	Gas
Dextrin	Ac: 9 Gas:189	Gas:15 Neg:1	Gas:14	Gas:12	Ac: 2 Gas:29 Neg:1	Gas:2	Ac: 2 Gas:9	Gas
Glycerol	Gas:198	Gas:16	Gas:14	Gas:12	Gas:32	Gas:2	Gas:11	Gas
Urea (slow)	Neg:8 Pos:190	Pos:16	Pos:14	Pos:12	Neg:3 Pos:29	Pos:2	Pos:11	Pos.
Urea (quick)	Neg:3 Pos:195	Pos:16	Pos:14	Pos:12	Neg:1 Pos:31	Pos:2	Pos:11	Pos.
Gelatin	Pos:29 Neg:169	Pos:5 Neg:11	Neg:14	Pos:2 Neg:10	Pos:27 Neg:5	Pos:1 Neg:1	Pos:6 Neg:5	Neg.
KCN	Pos:198	Pos:16	Pos:14	Pos:12	Neg:1 Pos:31	Pos:2	Neg:1 Pos:10	Pos.
Arginine	Pos:4 Neg:194	Neg:16	Neg:14	Neg:12	Neg:32	Neg:2	Neg:11	Neg.
Capsule	Pos:166 Neg:32	Pos:14 Neg:2	Pos:12 Neg:2	Pos:10 Neg:2	Pos:25 Neg:7	Pos:1 Neg:1	Pos:8 Neg:3	Neg.

Table 3. 202 *Cloaca* strains. All gas from cellobiose.

	177 motile forms					25 "O" forms			
Group	1	2	4	5	10	1	2	4	5
IMViC									
No. of strains	169	1	5	1	1	16	7	1	1
Dulcitol	Ac: 1 Gas:25 Neg:143	Neg.	Gas:2 Neg:3	Gas	Neg.	Gas:1 Neg:15	Ac: 1 Neg:6	Neg.	Neg.
Adonitol	Ac: 1 Gas:63 Neg:105	Neg.	Neg:4 Gas:1	Neg.	Neg.	Ac: 2 Neg:12 Gas:2	Neg:7	Neg.	Neg.
Inositol	Gas:27 Ac:71 Neg:71	Acid	Ac: 2 Neg:3	Acid	Gas	Gas:3 Ac:3 Neg:10	Ac: 1 Neg:6	Acid	Acid
Aesculin	Pos:55 Neg:114	Neg.	Pos:1 Neg:4	Pos.	Pos.	Pos:12 Neg:4	Pos:7	Pos.	Neg.
Starch	Gas:1 Ac: 49 Neg:119	Neg.	Ac: 1 Neg:4	Neg.	Acid	Ac: 2 Neg:14	Neg:7	Neg.	Acid
Dextrin	Gas:24 Ac:99 Neg:46	Gas	Gas:3 Neg:2	Neg.	Acid	Gas:1 Ac: 5 Neg:10	Ac: 3 Neg:4	Acid	Acid
Glycerol	Gas:27 Ac:62 Neg:80	Neg.	Ac:3 Neg:2	Acid	Gas	Gas:1 Ac: 11 Neg:4	Ac:7	Acid	Acid
Urea (slow)	Neg:169	Neg.	Neg:5	Neg.	Neg.	Neg:16	Neg:7	Neg.	Neg.
Urea (quick)	Neg:169	Neg.	Neg:5	Neg.	Neg.	Neg:16	Neg:7	Neg.	Neg.
Gelatin	Pos:154 Neg:15	Pos.	Pos:4 Neg:1	Pos.	Neg.	Pos:14 Neg:2	Pos:6 Neg:1	Pos.	Pos.
KCN	Neg:2 Pos:167	Pos.	Pos:5	Pos.	Pos.	Pos:16	Pos:7	Pos.	Pos.
Arginine	Pos:146 Neg:23	Pos.	Pos:4 Neg:1	Pos.	Neg.	Pos:14 Neg:2	Pos:4 Neg:3	Neg.	Pos.
Capsule	Pos:15 Neg:154	Pos.	Neg:5	Neg.	Neg.	Pos:1 Neg:15	Pos:1 Neg:6	Neg.	Neg.

plated, and we got positive and negative colonies. When both of these were replated the positive ones went on producing numerous negative variants, while the negative ones only produced a few or no positive colonies. It appears that gas production from starch is not a fixed character of this strain.

The tables also show that for the purpose of classification, gas production from starch, not acid productions alone, is of significance, as many motile Cloaca strains did produce acid. For comparison we have included in the tables our results with dextrin, which proved not to be as useful as starch. If we consider gas production from dextrin, 336 out of 355 Klebsiella cultures or 94 per cent were positive, but so were 28 of our 177 Cloaca strains, or 15 per cent; if we consider acid production we have only 2 negative Klebsiellae, or more than 99 per cent positive, but there were also 128 of 177 Cloacae positive, or 72 per cent. Therefore all we can say about dextrin is that in our opinion a culture in the family Enterobacteriaceae not fermenting it almost certainly is not Klebsiella.

When using the "slow test" for urea fermentation, we had in all 337 or 94 per cent of Klebsiella strains positive, and 344 or 96 per cent with our "quick test." No motile Cloaca culture was positive in either. Although the quick test gave better results we do not consider it as advisable for routine, as it involves more work; we reserve it for doubtful cases or when quick diagnosis is necessary.

In our opinion gelatin liquefaction does not deserve the importance that is attributed to it as one of the fundamental characters, if not the most important, in the differentiation of Klebsiella and Cloaca. It is true that no more than 17 or 9.6 per cent of our motile cultures were negative, but on the other hand we had 78 or 22 per cent positive nonmotile cultures which exhibited all the other characters of the Klebsiella group and which could not logically be put with the motile forms. It is interesting to note that IMViC group 6 of Klebsiella (+ - + +) was the one containing the highest percentage of gelatin liquefiers, as 3 out of 4 serotype strains and 27 of 32 of our own were liquefiers, in total 30 positive in 36, or 83 per cent. We could not observe any significant difference in the speed of liquefaction although in general Cloaca cultures were more active than Klebsiella; the shortest period was 3 days, observed with one culture of each group, but generally liquefaction was observable between 10 and 20 days.

Dulcitol was fermented by 123 out of 358 or 34 per cent of Klebsiella cultures and by 29 of 177 or 16 per cent of Cloaca strains, figures which are more in agreement with Moeller and with Edwards and Fife than with Brooke. As for adonitol we had 307 or 85 per cent positive Klebsiella and 65 or 3 per cent positive Cloaca. Salicin was fermented by all cultures of the two groups, even if a little slower by Cloaca; therefore in our experience these three carbohydrates are not useful for purposes of classification. In agreement with both Brooke and Moeller we found that 356 of our 358 Klebsiella cultures fermented inositol, almost 100 per cent, but 103 or 58 per cent motile cultures were also positive, in contrast with Brooke's results, and confirming the figures of Moeller and of Edwards and Fife. However, if gas production is considered, it will be seen in our tables that only 6 of the aerogenic Klebsiella cultures were negative, (98.4 per cent positive) whereas 28 out of 177 motile Cloaca strains were positive, or 15 per cent. As for arginine 152 or 85 per cent of 177 Cloaca cultures were positive against 5 positive Klebsiella in 358, or 1.4 per cent.

All aerogenic Klebsiella cultures produced gas from glycerol in 4 days, and in contrast only 28 or 15 per cent of Cloaca strains were gas-positive, which means that if this character alone had been considered, 85 per cent of them would have been rightly classified. We think therefore that the 3 characters, gas production from inositol and glycerol, and the arginine test are very useful, and when considering cultures isolated in Montevideo, they were especially useful as proved by the following analysis of our figures. From our 28 Cloaca cultures producing gas from glycerol, 23 also produced it from inositol and were arginine-negative. The remaining 5 behaved normally with respect to arginine, that is to say, were positive, and of these only one was also positive for gas in inositol, while 4 were negative. Two arginine negative cultures did not produce gas from either inositol or glycerol. It follows, therefore, that there is a group of Cloaca cultures, which for brevity we will call "type B", which combines the three unusual characters of producing gas from inositol and glycerol and being arginine-negative, and the correlation of these, although not perfect, is high.

Of these 23 "type B" cultures, 18 were among those we received from Dr. Edwards, and as mentioned before, we have included 43 of his strains in Table 3. This means that 41 per cent of them belonged here, while among the Montevideo

isolated cultures we found only 5 or 3.6 per cent. A possible explanation for the very different results obtained by Brooke working in Denmark and Edwards and Fife in the U.S.A. on gas production from glycerol by bacteria of the Cloaca group is that it may be due to different geographical distribution or source of isolation of the cultures tested by these authors. In any case the three tests were of considerable value to us as we had only 7.4 per cent glycerol or inositol gas-positive and less than 5 per cent arginine-negative locally isolated Cloaca cultures. Inulin was also tested with all cultures, but it proved of no interest, and we have only to mention that some klebsiellae fermented it, especially indole-positive strains.

The usefulness of the aesculin test is restricted to a few cases, but even so, this test may be of importance in confirming a doubtful diagnosis; only 1 of 286 Klebsiella cultures isolated in Montevideo was negative in contrast to 70 per cent of motile Cloaca strains. It will be observed though that there were 4 negative strains among the 72 Klebsiella serotypes. Capsules were observed in 82 per cent of our Montevideo Klebsiella cultures and (confirming Edwards and Fife) also in 9 per cent of those of motile Cloaca strains. All Klebsiella cultures, including our local strains and all of those of Cloaca, promptly fermented cellobiose and only 5 in a total of 569 tested cultures, or 0.8 per cent were KCN-negative. No indole-positive, motile or nonmotile Cloaca strains were found. We had 5 motile strains resembling the group, two of them with IMViC + + - + and 3 + + - -, which promptly fermented cellobiose with gas, some arginine or KCN-positive, but we think they are "intermediates" more closely related to Escherichia than to Cloaca on the basis of the other biochemical tests.

It may be interesting to mention the unusual behaviour of two strains not included in the tables. One of them, a nonmotile - - + +, capsulated, non-gelatin-liquefying, typical Klebsiella culture produced gas only in starch and dextrin, and transfers from these media to all other carbohydrates were constantly gas-negative; another, also a nonmotile, capsulated - + - +, urea-negative and arginine-negative culture, produced gas from adonitol, inositol, salicin, cellobiose, glycerol, starch, dextrin and other carbohydrates, but not from glucose, saccharose or mannitol, and all transfers from gas-positive tubes to these three carbohydrates were negative for gas. We think this strain should be considered as a urea-negative Klebsiella.

DISCUSSION

Although we are not prepared to arrive at final conclusions because the number of tested cultures is too small and also because geographical distribution may be of importance, as illustrated by the small number of "B type" Cloaca cultures isolated in Montevideo, we would like to emphasize some points which seem to us of practical value for routine work.

In our opinion cellobiose and KCN tests should be adopted by laboratories engaged in work on the Enterobacteriaceae. The first we found specially useful to differentiate cultures of anaerogenic, nonpigment-producing Serratia from Cloaca, a problem which frequently arises when dealing with strains isolated from human stools, bile, sputum or wounds. It is true that the first are arginine- and rhamnose-negative, but so are some strains of Cloaca; usually Serratia strains promptly liquefy gelatin (in one or two days) and peptonize milk, but when they do not cellobiose is important, as it is either not fermented by the group or very slowly. We have to add though, that among the aerogenic species of the group there are some, including at least some strains of S. marcescens, which promptly ferment cellobiose and several other carbohydrates with abundant gas, and that as their IMViC reactions are - - + + and they are KCN-positive, they could be very easily mistaken for strains of Cloaca if they did not produce pigment. We can confirm Moeller's statement about the usefulness of the KCN test, for differentiation of Escherichia coli from Klebsiella and Cloaca.

But even with all the tests we used we wonder if some of our strains really belong to either of the two groups. For instance, it could be that some motile cultures tentatively included in IMViC group 4 are more closely related to the Bethesda-Freundii group than to Cloaca inasmuch as loss-variant, non-H_2S producing strains of the first group could be very difficult to differentiate from Cloaca.

Since biochemical behaviour is accepted as the basis for defining the Enterobacteriaceae, we think that our results define the Klebsiella and Cloaca groups as follows:

Klebsiella. Nonmotile, promptly fermenting inositol and glycerol with gas, urea-positive, producing gas in 4 days from nonsoluble starch, arginine-negative and with the exception of some particular IMViC combinations, usually not liquefying gelatin.

Cloaca. Motile, usually not producing gas from inositol and glycerol (with the exception of the "B type"), urea-negative, generally arginine-positive and gelatin-positive and when fermenting nonsoluble starch not producing gas from it.

To these definitions should be added cellobiose fermentation and the KCN test, and mention should be made of the different IMViC combinations already known, as we do not see the point in calling so many strains "aberrant" that do not behave like group 1. It follows therefore that the 25 nonmotile cultures included in Table 3 have to be considered as Cloaca "O" forms since there is no character to differentiate them from the motile cultures in the same IMViC groups, although of course we do not know if they are variants derived from motile bacteria.

There is another consequence to which we would call attention, namely a culture in Table 1 in IMViC group 4 (- + - +); the "Ozena" capsular antigen 5 culture, Dr. Kauffmann's 5051. For the same reasons this particular strain has to be considered as an "O" form of Cloaca, since it is urea-negative, does not ferment starch and is positive in arginine, three of the most important characters of the group. This observation induced us to test more strains of this and of the 3, 4 and 6 serotypes, the two first included in Table 1 in groups 3 and 4 respectively of anaerogenic serotype cultures and the last in group 3 of the aerogenic ones, as none of them could be satisfactorily classified. We observed also that all of these and type 1 as mentioned before were the only Klebsiella strains showing delayed lactose fermentation, a character not infrequent among Cloaca cultures but which we have as yet never seen in other klebsiellae. For the purpose we employed 40 nonmotile capsulated strains serologically typed by Dr. Edwards which he kindly sent to us, all of them aerogenic, promptly fermenting cellobiose with gas, and aesculin-positive. We added two cultures of K. rhinoscleromatis which we obtained from the A.T.C.C., strains 6908 and 9436. The results were as follows:

1) Ten cultures of serological type 3, promptly fermented lactose and glycerol with gas and were arginine-negative. They could be divided into four groups.

a) Three cultures with IMViC reactions - + - +, KCN -, urea +, producing gas from inositol and starch, gelatin -

(2 strains), + (1 strain);

b) three with IMViC reactions - - + +, KCN +, urea +, producing gas from starch, gas from inositol (2), no gas from inositol (1), gelatin + (2), - (1);

c) three with IMViC reactions + - + +, KCN +, gelatin +, gas from inositol and starch, urea + (2), - (1); and

d) one with IMViC reactions + + - +, KCN +, urea +, gelatin +, gas from inositol and starch.

The two cultures from the A.T.C.C. were anaerogenic, their IMViC reactions were - + - -, they were lactose -, urea -, starch -, inositol +, glycerol +, aesculin +, gelatin -, arginine -. One of them grew weakly in KCN while the other did not grow.

The culture of Dr. Kauffmann's K. rhinoscleromatis 5046 is very similar to these two. Like them its IMViC is - + - -, it is glycerol and inositol +, urea - and arginine -, aesculin +, and grows poorly in KCN. It differs though in fermenting lactose and starch slowly, but as it is anaerogenic, this and other carbohydrate tests are of little use for classification.

In our opinion the two strains coming from the A.T.C.C. have to be considered as members of Cloaca or at least more closely related to this group than to Klebsiella as they are urea - and starch -. The systematic position of the type culture (originally NCTC 5046) seems to us rather doubtful and needs closer investigation. K. rhinoscleromatis is considered generally not only as a capsular serologic type but as a species recognizable by its biochemical characters. Among these Dr. Moeller says that all four decarboxylase tests are negative, and this is the only type which behaves in this way. In his paper he only mentions having tested one culture; on the other hand we have among our strains of Cloaca one motile and two "O" forms also negative in the four tests, which seems rather suggestive. Whatever the position of K. rhinoscleromatis, it is evident that the 10 strains of Dr. Edwards, although belonging to the same antigenic capsular group, are very different from it, and in our opinion have to be classified in Klebsiella.

2) Nineteen cultures of serotype 4 were divided into three groups.

a) Sixteen with IMViC reactions - - + + which behaved as Klebsiella. They were all gas positive in inositol,

glycerol and starch, promptly fermented lactose and split urea, and were arginine -; one grew poorly in KCN while the remaining 15 grew well, and 4 liquefied gelatin;

b) two strains with IMViC reactions - + - +, urea -, starch -, and gelatin -, fermented inositol with gas and differed in that one was arginine +, KCN - and fermented glycerol with gas, while the second was KCN +, arginine - and glycerol -;

c) one culture with IMViC reaction - - - +, fermented inositol and glycerol with gas, was arginine -, KCN -, urea -, starch - and gelatin -.

All three strains fermented lactose in 4 to 6 days, and we think they belong in Cloaca.

The serotype culture, Dr. Kauffmann's "Ozena" 5050 is another anaerogenic culture whose IMViC is - + - +, arginine - and gelatin -, grows weakly in KCN and ferments starch; as for urea, the slow test was positive and the quick test was negative. We do not think it possible to give an opinion about its taxonomic position, although it seems to be close to the two strains of Cloaca with the same IMViC mentioned in b).

3) Six cultures of serological type 5, all gelatin-negative and promptly fermenting lactose, divided into three groups.

a) One strain with IMViC - + - - did not ferment starch or dextrin, fermented glycerol without gas, produced gas from inositol, was urea -, arginine + and weakly positive in KCN;

b) three strains with IMViC - + - +, fermented inositol with gas, glycerol with acid only, two did not ferment starch, one produced acid only, all three fermented dextrin without gas, were urea - and grew poorly in KCN, one was arginine + and two were -;

c) two - - + + cultures with typical Klebsiella biochemical behaviour; gas production from inositol +, glycerol + and starch +, urea +, KCN + and arginine - .

As stated before in our opinion the serotype culture 5050 belongs to the Cloaca group and is very similar to the three strains mentioned in b) which differed from it only in that they did not produce gas from glycerol and that two of them were arginine - . As for the last two strains they completely differ from the serotype culture.

4) One culture of type 6, and 5 others serologically related, were all typical - - + + strains of Klebsiella, splitting urea, arginine -, producing gas from starch, glycerol and inositol, and KCN +. One liquefied gelatin. The systematic position of Dr. Kauffmann's F 5052, included in IMViC group 3, is not quite clear as it does not ferment urea and liquefies gelatin but produces gas from starch, glycerol and inositol, and is arginine -. We think it is probably an aberrant Klebsiella. All the 6 cultures tested differed from it.

When we compare the behaviour of all these strains with the respective serotype cultures, it is obvious that there is no correlation between capsular antigens and biochemical characters, a fact that was to be expected since Edwards has pointed out that some motile strains share the capsular antigens of Klebsiella, and it is known that they can even be found in the Escherichia group. Therefore for purposes of classification capsular typing cannot be used alone.

As was also to be expected there are some "intermediate" nonmotile cultures whose position cannot be satisfactorily decided as they share some characters of Klebsiella and of Cloaca. They do not seem to be of frequent occurrence since we did not find any among our own or Dr. Edwards' cultures, but only among serotype strains which constitute a highly selected material. Although a better knowledge of both groups is needed to solve the problem that these strains pose to the taxonomist, it is our opinion that the above mentioned tests should prove to be useful for routine work and in most cases sufficient to arrive at a correct classification.

As for the name to be given to the motile group, we feel inclined to agree with Dr. Edwards' suggestion that Aerobacter Cloaca should be adopted, with of course, the redefinition of that genus.

CONCLUSIONS

1) We suggest that the distinguishing characters of both groups, Klebsiella and Cloaca, should include cellobiose fermentation and KCN tests.

2) Separation of the two groups should be based on motility, urea hydrolysis, gas production from nonsoluble starch, inositol and glycerol, the arginine dihydrolase test and gelatin liquefaction, with mention of the frequency with

which the + - + + IMViC group of Klebsiella liquefy it. Reference should be made to the Cloaca "B type". The definition of both groups should also include the different known combinations of IMViC.

3) There is no correlation between capsular antigens and biochemical behaviour of the two groups so that serological typing cannot be used alone for identification of nonmotile cultures. Among 40 tested cultures of types 3, 4, 5 and 6, not one was exactly like the serotype culture, and there were also Klebsiella and Cloaca "O" forms.

4) The cultures of rhinoscleroma and ozena types 4 and 6 cannot be placed in Klebsiella or Cloaca without further investigation, as they seem to be "intermediates" between the two groups. In our opinion, the serotype of type 5 is an "O" form of a Cloaca.

5) We favour the suggestion of Edwards that the name Aerobacter should be chosen for the motile group; this would need the redefinition of the genus since the type species A. aerogenes disappears from it.

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Editorial Note.

The serotype strains referred to in this paper include those described by Kauffmann (based on NCTC 5046-5056, Goslings and Snijders types A-F), Brooke, Edwards and Fife, and Edmunds.

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SOME PHYSIOLOGICAL AND TOXIGENIC PROPERTIES OF MEMBERS OF THE GENUS MICROCOCCUS IN RELATION TO TAXONOMY[1]

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The controversy over the validity of the existence of the two genera Micrococcus and Staphylococcus (1, 7, 8, 9, 17) has been reopened recently. Resolution of the question had been attempted in the last edition of the Bergey Manual (5) by relegation of the genus Staphylococcus to a position of synonymy and definition of a series of species of Micrococcus to comprehend those of the two former genera closely in accord with the work of Hucker (12).

Apart from the question of the respective taxonomic legitimacy of retention of the two generic names (1), argument favouring recognition of the generic name Staphylococcus seems to centre upon a primary intent to reserve the generic name Staphylococcus for toxigenic forms of the non-chained Gram-positive micrococci. It has been suggested that the proposed genus be based upon the type species Staphylococcus aureus Rosenbach (for which the specific name pyogenes has also been preferred (8)) and for which culture F.D.A. 209 P (15) and, more recently, S33 R_4 (7) have each been proposed as the type-culture in the absence of an adequate record of a type-culture for the genus as originally propounded by Rosenbach (9).

A number of properties other than the "key" characteristics listed in the Bergey Manual have been described as being helpful aids in classification of this heterogeneous group, particularly with the objective of distinguishing between toxigenic (or pathogenic) and nontoxigenic forms. Gupta and Chakravarti (10) have recorded that pathogenic strains of the micrococci provide positive tests for both the methyl red and Voges Proskauer reactions while Evans, Bradford, and Niven (8) refer to "coagulase-positive staphylococci" as "one of the most homogeneous species of bacteria to be found in any genus" and recommend that this species be recognized as the type species for the genus Staphylococcus, a species said to be differentiable further by its property of the production of acid from glucose anaerobically in a defined complex medium.

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[1]A contribution from the Microbiology Section of the Laboratories of the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Barber and Kuper (3) and Carrère and Roux (6) have offered evidence to show that the production of phosphatase by micrococci may be used as an indicator of potential pathogenicity. Further, Cowan, Shaw and Williams (7) have recently used an argument based on phage relationship to reverse a proposal of Shaw, Stitt and Cowan (15) that strain F.D.A. 209 P be considered as the neotype for a proposed species Staphylococcus aureus.

As a contribution to this question of nomenclature and in order to explore any possible relationship between the various properties of micrococci referred to above we have determined the following properties from a collection of 263 cultures of micrococci isolated from clinical sources and from foods: (a) their specific nomenclature in accord with the primary "key" properties to the species of the genus Micrococcus as listed in Bergey, viz.; formation of acid from mannitol, production of pigment, the use of ammonium phosphate as a sole source of nitrogen in a standard medium, the reduction of nitrate to nitrite, the liquefaction of gelatin, and litmus-milk reactions; (b) the methyl red and Voges Proskauer reactions; (c) anaerobic production of acid from glucose; (d) the production of coagulase and phosphatase; (e) the production of alpha and beta hemolysins and enterotoxin, (f) the phage relationships of selected representative cultures.

METHODS

The test cultures used in this study, unless otherwise indicated, were originally selected from strains that produced acid on mannitol-salt agar (Difco). The biochemical and cultural properties as referred to in Bergey (5) were determined for all cultures in accord with the Manual of Methods for Pure Culture Study (14). The production of acid from glucose under anaerobic conditions was determined using the medium and method described by Evans, Bradford and Niven (8) which involved use of a supernatant seal of "vaspar" to obtain anaerobiosis, and for corroboratory purposes, the same medium incubated under rigidly anaerobic conditions in Brewer jars in an atmosphere of hydrogen. Coagulase, phosphatase and the three specific toxins were determined as described by Thatcher and Simon (16). Phage-typing was carried out by standard methods at the National Phage-typing Centre, Ottawa, through

the courtesy of Dr. E. T. Bynoe. The determination of toxins and phage reactions was limited to fifty cultures randomly selected from within groups of cultures demonstrated to have specific properties of coagulase and phosphatase production, viz., in the respective categories + +, + -, - + and - - for the two enzymes.

RESULTS

The data shown in Table 1 reports the distribution of 256 mannitol-positive and 7 mannitol-negative cultures within the species of Micrococcus (after Bergey), and lists their "key" reactions, the "MR-VP" reactions, their capacity to grow anaerobically and the pH of the culture medium after incubation for 10 days at room temperature under strictly anaerobic conditions. Ten species were recognized, though specific assignment in some instances could not be entirely free from equivocation. The species M. pyogenes was represented by 173 cultures of which 82 were of the variety aureus and 91 the variety albus.

Representatives of seven species gave positive reactions for both the MR and VP tests, and representatives from all species were able to grow anaerobically with production of acid under the conditions defined by Evans, Bradford, and Niven (8). This latter result was duplicated when anaerobiosis was effected by the use of hydrogen in Brewer jars equipped with a sensitive Eh indicator (14) that remained in reduced condition throughout the incubation period.

Subsequent to completion of the foregoing experiment the authors learned that Breed (4) has recently endorsed the suggestion of Evans et al. (8) that the anaerobic production of acid from glucose should be a determinative criterion for the genus Staphylococcus. Because of the demonstration described above of the common occurrence of this property among species normally considered to be nonpathogenic, we have redetermined the production of acid aerobically and under the two conditions of anaerobiosis previously described from all cultures used in this study. Resultant pH values are expressed in Table 2. The original findings are confirmed.

Table 3 reports the distribution of cultures within each species based on their capacity to produce coagulase and phosphatase. The only species able to produce both enzymes

TABLE I

Determinative Reactions of Representatives of Various Micrococcus Species.

Micrococcus Species (After Bergey, 6th Ed.)	No. of Cultures	DETERMINATIVE REACTIONS									
		Mannitol	Pigment	Gelatin	Litmus Milk	Ammonium Phosphate	Nitrite	M.R.	V.P.	Anaerobic Growth	pH[1]
pyogenes v. aureus	82	+	orange	+	acid, coag.	-	+	+	+	+	4.92 (5.20 - 4.80)
pyogenes v. albus	91	+	white	+	acid, coag.	-	+	+	+	+	4.96 (6.50 - 4.70)
aurantiacus	24	+	orange	-	acid only	-	+	+	+	+	5.18 (5.72 - 4.84)
candidus	23	±	white	-	acid only	-	-	+	+	+	5.01 (6.03 - 4.73)
freudenreichii	10	±	white	+	acid, coag.	+	-	+	-	+	4.94 (5.77 - 4.12)
conglomeratus	5	±	yellow	+	acid only	+	+	-	-	+	5.40 (6.72 - 4.92)
caseolyticus	5	+	variable	+	acid, coag. pept.	+	-	+	+	+	5.01 (5.90 - 4.18)
flavus	14	±	yellow	+	acid, coag.	+	-	±	±	+	4.98 (5.80 - 4.70)
varians	1	+	variable	-	acid, coag.+	+	+	-	-	±	5.02
ureae	1	+	white	-	N.C., alk.	+	-	+	+	+	*
epidermidis	7	-	white	-	acid only	-	+	+	+	+	4.74 (5.64 - 4.18)

[1] Average values thus - 4.92
Range of values thus - (5.20 - 4.80)

* Acid by indicator; pH not recorded

TABLE 2

The Production of Acid
Aerobically and under two conditions of anaerobiosis
by representatives of pathogenic and nonpathogenic species of Micrococcus

Species	pH after standard culture: average and range values		
	Aerobic	Anaerobic (Vaspar)	Anaerobic (Brewer jar)
M. pyogenes var. aureus	4.87 (5.10-4.77)	4.83 (4.95-4.69)	4.71 (4.98-4.53)
M. pyogenes var. albus	5.15 (5.72-4.80)	5.03 (5.38-4.72)	5.00 (5.45-4.66)
M. aurantiacus	5.12 (5.27-4.96)	5.26 (5.98-4.93)	5.18 (5.72-4.84)
M. freudenreichii	4.92 (5.27-4.15)	4.77 (5.00-4.22)	4.94 (5.77-4.12)
M. candidus	5.12 (5.55-4.55)	5.30 (5.80-4.75)	5.34 (6.03-4.73)
M. conglomeratus	5.88 (7.25-5.10)	5.42 (6.89-4.94)	5.40 (6.72-4.92)
M. varians	5.25	4.95	5.02
M. flavus	5.20 (5.50-4.80)	5.18 (5.80-4.93)	5.11 (5.81-4.72)
M. caseolyticus	5.05 (6.15-4.20)	4.90 (5.84-4.28)	5.01 (5.90-4.18)
M. epidermidis	4.74 (5.22-4.20)	4.76 (5.61-4.26)	4.74 (5.64-4.18)
Control	6.90	6.90	6.61

was M. pyogenes. Of 82 representatives of M. pyogenes var. aureus, 70 were in the + + reaction category for both coagulase and phosphatase, 9 were in the respective - + category, and three failed to produce either enzyme. Of the 91 cultures of M. pyogenes var. albus, 30 were in the + + category, 39 in the - +, 4 in the + -, and 18 in the - - category. The production of coagulase was confined to the species M. pyogenes but not as a consistent characteristic of the species since only 60 per cent of the cultures within the two varieties aureus and albus produced this enzyme. Among the aureus cultures, 85 per cent produced coagulase with the corresponding value for the albus cultures being 37 per cent.

Phosphatase was detected not only in cultures of the species M. pyogenes but also in the species M. aurantiacus, M. candidus, M. freudenreichii, M. caseolyticus, M. flavus and M. epidermidis.

The data expressing the production of specific toxins from the 50 cultures randomly selected from the various coagulase-phosphatase categories as described earlier as shown in Table 4. Alpha-lysin was produced consistently by all 23 representatives of the + + category. Twelve produced alpha and beta lysins and enterotoxin. However, all possible variations for the production of beta lysin and enterotoxin are indicated. Among the 15 cultures in the - + category, 9 failed to produce any of the three toxins, one produced all three toxins, 6 produced enterotoxin. Only one culture in this group produced beta lysin. Only two cultures were found in the + - coagulase-phosphatase category. One produced all three toxins, the other produced none. Among the 10 cultures in the - - category, very low titres of alpha lysin were produced by 4 of them, one produced enterotoxin; one produced beta lysin (titre 1:4) and 6 were without detectable toxins.

The species relationships of this selected group and the phage group to which they belong are indicated in Table 5 together with the toxin production by the representatives of the various species. Clearly, the greatest propensity for the formation of toxins is possessed by the species M. pyogenes, but toxins were also produced by representatives of four other species. All representatives of phage group IV produced all three toxins, whereas a wide variation of toxin production is shown by members of phage groups I, II and III. All strains lysed by the standard phages were members of the species M. pyogenes, but 10 of 31 isolates of this species were not susceptible to lysis by the phages. Within this "non-typable" group (all of which were coagulase-negative)

TABLE 3

Coagulase and Phosphatase Production
by representatives of various species[1] of *Micrococcus*

Species	Number of Cultures	Reaction Group	
		Coagulase	Phosphatase
M. pyogenes var. *aureus*	70	+	+
	9	-	+
	3	-	-
M. pyogenes var. *albus*	30	+	+
	39	-	+
	4	+	-
	18	-	-
M. aurantiacus	4	-	+
	20	-	-
M. candidus	6	-	+
	17	-	-
M. freudenreichii	2	-	+
	8	-	-
M. caseolyticus	1	-	+
	4	-	-
M. flavus	3	-	+
	11	-	-
M. epidermidis	3	-	+
	4	-	-

[1] After Bergey, 6th Edition.

TABLE 4

Specific Toxin Production[1] by Strains of Micrococcus
Coagulase-Phosphatase Reactions

Number of Cultures	Coagulase	Phosphatase	α-Lysin	β-Lysin	Enterotoxin[3]
12	+	+	+	+	+
7	+	+	+	-	+
1	+	+	+	+	-
3	+	+	+	-	-
1	-	+	+	+	+
3	-	+	+	-	+
2	-	+	-	-	+
9	-	+	-	-	-
1	+	-	+	+	+
1	+	-	-	-	-
1	-	-	+ (1:2)[2]	-	+
1	-	-	+ (1:2)	+ (1:4)	-
2	-	-	+ (1:8)	-	-
6	-	-	-	-	-

[1]Toxins determined from cultures randomly selected in accord with the various coagulase-phosphatase reactions.
[2]Maximum titre.
[3]Emesis in cats from intraperitoneal injection of 2 ml. filtrate per kilo.

TABLE 5

Toxin Production by Selected[1] Strains of *Micrococcus*
In relation to species and phage group

Species	Phage Group	No. of Cultures	α-Lysin	β-Lysin	Enterotoxin
M. pyogenes, varieties *aureus* and *albus*	I	1	+	+	-
	I	1	+	-	-
	II	1	+	+	+
	II	1	+	-	+
	III	2	+	+	+
	III	1	+	+	-
	III	1	+	-	-
	III	5	+	-	+
	IV	8	+	+	+
	N.T.[2]	1	+	+	+
	N.T.	2	+	-	+
	N.T.	5	-	-	-
	N.T.	1	-	-	+
	N.T.	1	+	+	-
M. aurantiacus	N.T.	1	+[3]	-	+
	N.T.	1	+[3]	-	-
M. freudenreichii	N.T.	1	+[3]	-	+
M. conglomeratus	N.T.	1	+[4]	-	-
M. epidermidis	N.T.	1	-	-	+

[1] Randomly selected from groups of cultures of different coagulase-phosphatase reactions as specified in text.
[2] N.T.--nontypable by standard phages.
[3] Maximum titre--1:4.
[4] Maximum titre--1:8.

5 cultures produced toxins. The cultures of the remaining species were all coagulase-negative and "non-typable" but included strains that produced alpha lysin at low titre as well as enterotoxin.

DISCUSSION

The foregoing results point to the marked genetical heterogeneity of the tested cultures of micrococci: a heterogeneity that is expressed in terms of the simple determinative characters, in coagulase and phosphatase production, in specific toxigenicity, phage susceptibility and in interrelationships between these properties.

The key to the genus Micrococcus as listed in the Bergey Manual gives primary significance to the production of pigment and to liquefaction of gelatin in distinguishing between a number of species, while pigment production is the essential difference between the varieties aureus and albus of M. pyogenes. While we have not recorded the data in this paper, our experience has been in accord with that of other workers who have found the production of pigment by M. pyogenes to be subject to variation with the most common trend being for golden coloured strains of M. pyogenes to give rise to colourless or white strains; while it is our belief that the continued growth of certain white strains in a pathogenic environment (as, for instance, when inoculated into the chick embryo) leads on occasion to the development of golden strains. The colour change in this direction has been established also by Barber (2) and by Lack and Wailling (13). Thus, the retention of two varieties of this species on the basis of pigmentation alone seems to be invalid. Comparable reasoning may well apply to the distinction of M. citreus which differs from M. pyogenes in its key characteristics in pigment only, though it is recognized that the expanded description of species in the Bergey Manual offers other distinguishing properties. This applies to other species discussed here.

Similarly, a metabolic change in the ability to liquefy gelatin could also cause interspecific confusion, for if a golden strain of M. pyogenes were to lose ability to liquefy gelatin it would then more closely resemble the species M. aurantiacus; while, if the statement of Evans, Bradford

and Niven (8) be accepted that growth in ammonium phosphate medium is dependent on the vitamin content of the inoculum, then change in gelatin response in an albus variant of M. pyogenes would render it subject to classification as M. varians.

The data in this paper establish that neither the MR-VP reactions of Gupta and Chakravarti nor the anaerobic production of acid from glucose are exclusively related to groups of cultures with distinct homogeneity. Instead these properties are to be found among representatives of several species as presently defined, while neither reaction is related dependably to toxin production, which, in turn, is not a restrictive function of those strains that produce either coagulase or phosphatase.

The authors are perplexed by the conclusion of Evans et al. (8) that, "The genera Micrococcus and Staphylococcus can be separated logically on the basis of their relation to oxygen in a standard complex medium containing glucose", for Table 1 of their paper shows that while M. pyogenes (85320) produces acid to provide a final pH of 5.6 under aerobic conditions and pH 5.0 anaerobically, M. caseolyticus produces acid to provide a final pH of 5.0 under both conditions, M. epidermidis provides the respective values of pH 5.3, and 5.1, and M. candidus 4.6 and 4.3. Our data on acid production by these same species are in close accord with those of Evans et al.: average pH after anaerobic incubation for 82 cultures of M. pyogenes var. aureus was 4.92; for 91 cultures of M. pyogenes var. albus, 4.96; for 23 cultures of M. candidus, 5.01; for 5 cultures of M. caseolyticus, 5.01; and for 7 cultures of M. epidermidis, 4.74. Variation in the capacity to produce acid is also apparent within a species, as shown by a final pH range for M. pyogenes of 6.50 to 4.70. Consistency in interpretation of the suggestion of Evans et al. would seem to require that the three species last named be included in the genus Staphylococcus. But each of these three species is considered to be normally nonpathogenic. Further, our data show that certain strains of other nonpathogenic species are able to grow and to produce acid from the test medium under anaerobic conditions. We find it difficult to see any advantage in establishing a new genus which would need to include strains of at least the ten species listed in Table 1 and which would fail to provide any better recognition of pathogens than at present available.

The coagulase reaction is a useful exploratory indicator of toxigenicity and seems to be limited to the present species M. pyogenes, but not all isolates of this species as currently defined are coagulase-positive. Neither is toxigenicity a property of all members of M. pyogenes nor even of its coagulase-positive representatives. In particular, we have recorded enterotoxin from coagulase-negative strains and from a number of other so-called species. This would be in accord with the work of Haynes and Hucker (11), and is further supported by our observation (16) that a sub-culture of an "albus" variant (12069 α) originally isolated by Dolman from a former golden strain of M. pyogenes var. aureus that had been implicated in an episode of food poisoning is now, after several years of culture, coagulase-negative, colourless, nontypable by phage but still produces its characteristic toxin pattern, viz. alpha lysin but no beta lysin and enterotoxin. We have also noticed (16) that certain cultures have been coagulase-negative for as many as three tests conducted shortly after isolation from clinical material, but after a number of sub-cultures on an agar medium have developed the capacity to produce coagulase and have been phage-typable. Two such strains were shown to cause emesis in monkeys.[1]

In our hands the production of phosphatase has been a less reliable indicator of toxigenicity of a given strain than is the formation of coagulase. Phosphatase was detected among five species not usually considered pathogenic and was frequently produced in the absence of either of the three toxins for which tests were made.

The phage determinations from the selected group of cultures described in this work show a striking consistence by members of phage group IV in the production of all three toxins. Further, this group in general produces toxins at titres higher than for other phage groups. Within the experience of this laboratory a member of phage group IV has never been found which did not produce a high titre of lysins. Further, the only variant factor we have encountered among this group has been in pigment production. Biochemically determined properties have seemed to be stable to a marked degree.

[1]Through the courtesy of Dr. C. E. Evans, American Meat Institute, Chicago.

These last observations are pertinent to a decision of Cowan, Shaw and Williams (7) to reverse a proposal of Shaw, Stitt and Cowan (15) that strain F.D.A. 209 P be considered as the "neotype" or "type strain" for a proposed species Staphylococcus aureus. The earlier proposal was changed because strain F.D.A. 209 P was found to be lysed by phage 42 D and therefore a member of Group IV, considered to be of bovine origin. The data reported in this present paper seem to warrant the suggestion that a decision on this basis should be re-examined, because members of Group IV, due to their marked constancy of determinative characters and of multiple production of toxins might well be more truly a parental "clone" and genetically more suitable as a source of an arbitrarily selected neotype strain from which others may show varying degrees of change. A wide range of variance is particularly evident among members of phage group III from which the newly proposed neotype has been chosen, the choice being based upon the numerical preponderance of Group III among clinical specimens.

GENERAL CONCLUSIONS

Based on the observations expressed above and in response to the request of the Editorial Board of this Bulletin (1) the following recommendations are offered:

1. Postulation of the two genera Micrococcus and Staphylococcus is not yet warranted on a taxonomic basis because, at present, no scheme of consistent determinative properties of micrococci has been described for positive recognition of distinctive groups of generic rank. The evidence points to pronounced genetical heterogeneity among the cocci under discussion.

2. Variation in pigment production, being on occasion reversible, offers a dubious basis for taxonomic distinction between varieties of the species M. pyogenes.

3. If it be deemed desirable to separate pathogens from nonpathogens and to establish two genera by arbitrary postulation, then a majority of pathogens may be recognized on the basis of susceptibility to standard phages which at

present seems to be limited to coagulase-positive strains. However, neither the coagulase reaction nor phage susceptibility seems to be an absolute criterion of distinction for toxigenic strains, and the latter property has practical objections in that it involves procedures not widely available to the smaller laboratories; while if coagulase-production were adopted as a primary specific character this would result in exclusion from the proposed species, S. pyogenes, several strains that are M. pyogenes by present criteria.

If phage susceptibility were to be considered as a determinative factor in any such arbitrary segregation of strains then choice of a member of a highly variable group (Group III) as a neotype seems inadvisable when some acceptance already has been extended to a well-defined and widely distributed member of a group that seems to be genetically more stable and is consistently productive of known toxins. If this argument be accepted, then strain F.D.A. 209 P would constitute a preferable neotype as compared with strain S33 R_4, even though the former is a representative of a phage group most commonly encountered among bovines and dairy products.

4. The demonstrated heterogeneity among the micrococci leads to a concept of a group of strains of bacteria whose differing representatives possess a high degree of "overlapping" of characters and among which transitional, and to some extent genetically unstable, forms make species differentiation more than usually arbitrary. Restudy of existing schemes for distinguishing species seems desirable in the light of such a concept, an evaluation which seems to parallel the suggestion of Barber (2).

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STATUS OF THE GENUS CHROMOBACTERIUM

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Papers have been published during the past year on the status of the genus Chromobacterium by Leifson (2) (Morphological and physiological characteristics of the genus Chromobacterium); by Sneath (3) (Cultural and biochemical characteristics of the genus Chromobacterium); by Sneath (4) (The change from polar to peritrichous flagellation in Chromobacterium spp.); by Eltinge (1) (Nitrate reduction in the genus Chromobacterium) and by Sneath (5) (On conservation of the genus name and designation of the type species and type strains).

Detailed studies of the flagellation in this genus have been carried out by both Leifson and Sneath. They agree that all strains studied have a polar flagellum and may have in addition one or more lateral flagella. They also found that the lateral flagella have a shorter wave length and are more easily stained than the polar ones. Sneath concluded that both cultural conditions and age of the culture largely determined the presence or absence of lateral flagella. These were most abundant in young cultures on solid media and least abundant in broth cultures and in old cultures on solid media. Sneath also found that the two types of flagella differed in antigenic composition.

Either of two methods may be chosen for a study of the physiological differences between organisms belonging to a genus. By one method numerous strains may be assembled from culture collections and others isolated over a period of years, from as many localities as possible, and studied by as many tests as time and facilities permit. Or alternatively fewer cultures may be collected and intensively studied using many more differential criteria and tests. Both methods have value, and when critical studies of selected strains are combined with less comprehensive studies of a large number of strains, noteworthy differences should become apparent.

Leifson and Sneath made use of the second method in studies of 28 and 38 cultures respectively. Their cultures indicated that the genus could be divided into psychrophiles and mesophiles with a group of other characteristics common

to each of these groups. Eltinge also thought the genus should be divided on temperature until she obtained three cultures isolated from soil from the southern states which were mesophiles but had all other characteristics which are common to psychrophiles. She has also observed that a strain which usually produced good growth at 4° C might at times be unable to grow at 4° C and still not be able to grow at 37° C. She concluded that temperature does not seem to be a wholly satisfactory criterion upon which to base differentiation of species.

Eltinge in a study of 82 cultures concluded that the genus may be divided into three groups of strains on the basis of ability to reduce nitrate. One group reduces nitrate completely either with or without the production of visible gas. This group includes the psychrophiles and the three mesophiles isolated from southern soil. Strains in the second group reduce nitrate to nitrite but are not able to reduce nitrite. Those in the third group are unable to reduce either nitrate or nitrite. Based on other common characteristics, the second and third groups may well be combined. Results reported by Sneath are not completely in harmony with this grouping on the basis of nitrate reduction. He found most mesophiles could utilize nitrite slightly and that an occasional strain could do so rapidly.

Leifson reported that some of the mesophiles, which oxidized glucose, were also able to ferment glucose and a few other carbohydrates anaerobically. This finding has been confirmed by both Sneath and Eltinge. Leifson would separate this group of fermenters from other mesophiles which can oxidize only. This finding would make plausible the recognition of two species in the mesophilic group.

When tests on carbohydrate utilization as reported respectively by Leifson, Sneath and Eltinge are compared, some variation in results are evident. These could doubtless be caused by adaptations within the strain, perhaps permanent or transitory caused by environmental conditions. In the six years the writer has worked with members of this genus she has found many strains capable of varied adaptation. Sometimes cultures of presumably identical strains obtained from different individuals will differ in one or more characters when tested.

Another criterion available for separation of species within the genus is utilization of maltose. So far, this seems a dependable distinguishing characteristic. Strains

able to utilize maltose include all the psychrophiles and the three strains from southern soil. These are also the strains which completely reduce nitrate. All other strains seem unable to use maltose. However, recognizing how much variation there may be in utilization of carbohydrates between strains, a recognition of species within the genus based solely upon the utilization of a specific carbohydrate does not seem wise.

The three authors agree that the genus name for the violet organisms with pigment soluble in ethanol but not in chloroform or water should be Chromobacterium. Leifson, Sneath and Eltinge agree that in Chromobacterium there should be recognized at least two species, but they do not agree as to the criteria. Leifson and Sneath would divide the genus on temperature and Eltinge upon the utilization of nitrate. Sneath would make two species. He has proposed the name Chromobacterium violaceum Bergonzini 1880 for mesophilic strains and Chromobacterium lividum (Eisenberg) Holland for psychrophilic strains. Sneath (5) recommended that the type species be a mesophilic one.

Leifson would recognize three species. He proposes that the name Chromobacterium violaceum (Schroeter) Bergonzini be assigned to the psychrophilic strains. He would make two species of the mesophilic strains based on differences in carbohydrate utilization. For strains able to ferment he proposed the name Chromobacterium manilae comb. nov. and for those able to oxidize only the name Chromobacterium laurentium comb. nov.

The author would make two species. The name Chromobacterium violaceum (Schroeter) Bergonzini is proposed for strains able to reduce nitrate completely and which usually grow at 4° C if a psychrophile is chosen as the type species, otherwise the name C. lividum (Eisenberg) Holland is proposed for the species. She would also recommend in addition to the typical variety, a named variety for the strains able to grow at 37° C and not at 4° C and suggests the new name Chromobacterium violaceum var. mesophilum. For strains which fail to reduce nitrate or reduce it only to nitrite, which grow at 37° C or higher and which usually ferment some carbohydrates she proposes the name Chromobacterium janthinum (Zopf) Holland; if the mesophile is chosen as the type species she proposes instead the name C. violaceum Bergonzini. It may be that two varieties should be recognized, one based upon ability to reduce nitrates to nitrites. Another possibility

is the recognition of two varieties based upon ability to ferment carbohydrates.

The name of the type species of a genus must under the rules be the name of a species included by the author in the genus when first proposed. One species only was named by Bergonzini in the original paper. The name Chromobacterium violaceum Bergonzini must therefore be applied to the type species. The question arises as to whether this name shall be applied to a mesophilic or a psychrophilic strain. What names should be applied to other species is also debatable.

Sneath proposes that the type species be fixed as a mesophile. The author agrees with him. One reason for this is that a mesophilic strain lasts longer under cultivation than a psychrophilic one and therefore, is more often found in culture collections. If a mesophilic strain is chosen as the neotype culture or strain, then the strain MK of Sneath (NCTC 9757, ATCC 12472) would be suitable. If a psychrophilic strain is chosen as the neotype, then the strain HB of Sneath (NCTC 9796, ATCC 12473) is suitable.

The views of Leifson, Sneath and the author as to the differential characters and species names to be applied in the genus Chromobacterium may be summarized by the following three keys.

Chromobacterium

Key to species according to Leifson (2).

I. No growth at 37° C. Oxidative metabolism of carbohydrates. Acid from glucose, levulose, mannose, arabinose, xylose, maltose, mannitol, and sorbitol. Sucrose variable. Lactose, salicin and dulcitol negative. Nitrite produced from nitrate. Utilization of citrate positive. Gelatin liquefaction weak or negative. Indole and urea negative.

1. Chromobacterium violaceum (Schroeter) Bergonzini.

II. Growth at 37° C.

A. Fermentative metabolism of carbohydrates. Acid without gas from glucose, levulose, mannose and inulin. Sucrose variable. Arabinose, xylose, maltose, mannitol, sorbitol, lactose, salicin and dulcitol negative. Nitrate reduced to nitrite or negative. Gelatin liquefied. Citrate utilization variable. Indole and urea negative.

2. Chromobacterium manilae Leifson.

B. Oxidative metabolism of carbohydrates. Acid from glucose and levulose. Mannose, arabinose, xylose, lactose, sucrose, maltose, inulin, mannitol, sorbitol, salicin and dulcitol negative. Nitrate reduced to nitrite. Gelatin liquefied. Citrate negative or slowly metabolized. Indole and urea negative.
3. Chromobacterium laurentium Leifson.

Chromobacterium
Differentiation of species according to Sneath (3, 4, 5).

I. Strains which grow at 4° C but not at 37° C. Size large, fat scanty, proteolysis slight, haemolysis slight, anaerobic growth scanty or absent, citrate utilized rapidly, HCN not detected, egg yolk reaction negative, oxidizes glucose, does not break down trehalose, hydrolyzes aesculin.
1. Chromobacterium lividum (Eisenberg 1891) Holland 1920.

II. Strains which grow at 37° C but not at 4° C. Size small, fat usually abundant, proteolysis marked, haemolysis marked, anaerobic growth usually good, utilize citrate slowly, produce HCN, egg yolk reaction positive, acid from trehalose, aesculin not hydrolyzed, attack on glucose usually fermentative.
2. Chromobacterium violaceum Bergonzini 1880.

Chromobacterium
Key to species according to Eltinge.

(1) If a psychrophile is chosen as the type species.

I. Nitrate reduced completely with or without the production of visible gas. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose. Gelatin slowly liquefied. Usually good growth at 4° C with no growth at 37° C.
1. Chromobacterium violaceum (Schroeter) Bergonzini.

A. Strains which grow at 4° C but not at 37° C. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose.
1a. Subspecies (var.) violaceum var. nov.

B. Strains which grow at 37° C but not at 4° C. Oxidize glucose and maltose and occasionally fructose.
1b. Subspecies (var.) mesophilum var. nov.

II. Nitrate reduced to nitrite or not at all. Oxidize glucose, frequently fructose and occasionally sucrose. May ferment glucose, sucrose, fructose and arabinose. All grow well at 37°, many at 40° and a few at 42° C. No growth at 4° C. Gelatin rapidly liquefied.
2. C. janthinum (Zopf) Holland.

A. Strains which ferment glucose and sucrose, frequently fructose and occasionally arabinose. Oxidize glucose, frequently fructose and occasionally sucrose. May or may not reduce nitrate to nitrite.
2a. Strains which reduce nitrate to nitrite--subspecies (var.) janthinum var. nov.
2b. Strains which do not reduce nitrate--subspecies (var.) anitritum var. nov.

B. Strains which oxidize glucose and usually fructose but do not ferment carbohydrates. Nitrates reduced to nitrites.
2c. Subspecies (var.) purpureum var. nov.

(2) If a mesophile is chosen as the type species.

I. Nitrate reduced completely with or without the production of visible gas. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose. Gelatin slowly liquefied. Usually good growth at 4° C with no growth at 37° C.
1. Chromobacterium lividum (Eisenberg 1891) Holland 1920.

A. Strains which grow at 4° C but not at 37° C. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose.
1a. Subspecies (var.) lividum var. nov.

B. Strains which grow at 37° C but not at 4° C. Oxidize glucose and maltose and occasionally fructose.
1b. Subspecies (var.) mesophilum var. nov.

II. Nitrate reduced to nitrite or not at all. Oxidize glucose, frequently fructose and occasionally sucrose. May ferment glucose, sucrose, fructose and arabinose. All grow well at 37° C, many at 40° C and a few at 42° C. No growth at 4° C. Gelatin rapidly liquefied.
2. C. violaceum Bergonzini 1880.

A. Strains which ferment glucose and sucrose, frequently fructose and occasionally arabinose. Oxidize glucose, frequently fructose and occasionally sucrose. May or may not reduce nitrate to nitrite.
2a. Strains which reduce nitrate to nitrite--subspecies (var.) violaceum var. nov.
2b. Strains which do not reduce nitrate--subspecies (var.) anitritum var. nov.

B. Strains which oxidize glucose and usually fructose but do not ferment carbohydrates. Nitrates reduced to nitrites.
2c. Subspecies (var.) purpureum var. nov.

SUMMARY

Chromobacterium (like many other bacterial genera) is difficult to divide into species because of the considerable differences between strains. In conclusion it should be pointed out that neither temperature nor reduction of nitrate seem to be the perfect criterion for delimitation of species. There are intermediates. However, most strains may easily be separated into two groups either on the basis of temperature or on nitrate reduction. When 82 strains were compared, a division based on nitrate reduction seemed to give more consistent results than one based on temperature.

All three authors agree on the use of Chromobacterium violaceum to designate one of the species. Two agree that this name should be given to a psychrophile, the third to a mesophile.

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NEWS AND NOTES

RESIGNATIONS AND APPOINTMENTS, ENTEROBACTERIACEAE SUBCOMMITTEE. Brig. J. S. K. Boyd, and Lt. Col. H. J. Bensted have resigned from the Enterobacteriaceae Subcommittee.

Mrs. K. P. Carpenter, Dysentery Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London N. W. 9, England, has been newly elected to membership in this subcommittee.

WANTED: A WORD. Dr. S. T. Cowan of the National Collection of Type Cultures, London, in a recent meeting of The Society for General Microbiology, discussed the problem of finding a suitable shorter name for the INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY. He suggests the possibility of coining or adapting a Greek compound. For example, from the Greek onoma (combining form onomat-) he proposes ONOMATOTAXY to include nomenclature and taxonomy, also the derivatives ONOMATOTAXOLOGY, ONOMATOTAXOLOGIST, ONOMATOTAXIC, and others. Perhaps the BULLETIN could be renamed BACTERIAL ONOMATOTAXY, or ONOMATOTAXOLOGY.

Editorial Note. The Board of Editors are in agreement that the name INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY is too long and is not satisfactorily descriptive of scope and purpose. It is hoped that with the beginning of the year 1958 the format of the BULLETIN may be materially improved; if a better designation can be evolved this would be an appropriate time to initiate the change in name. As noted by Dr. Cowan, suggestions for a more satisfactory name will be welcomed.

Several revisions have been proposed, among them are: BULLETIN OF SYSTEMATIC BACTERIOLOGY, SYSTEMATIC BACTERIOLOGY, JOURNAL OF SYSTEMATIC BACTERIOLOGY, SYSTEMATIC MICROBIOLOGY.

There are several journals in related fields. The international botanical organ is TAXON, the Society of Systematic Zoology issues SYSTEMATIC ZOOLOGY, and the American Society of Plant Taxonomists edits THE TAXONOMIC INDEX. Other journals in botany which deal with botanical taxonomy in somewhat more limited fields have been named from plant genera, as RHODORA, from the non-Latin name of a plant as Madroño, or to honor a botanist, as BRITTONIA.

One recognizes that Dr. Cowan's ONOMATOTAXY is TAXONOMY in reverse. Words of Greek origin used in English include

ONOMASTICON, a vocabulary or collection of names; ONOMATOLOGY, the science of names, terminology; ONOMATOLOGIST, a student of names.

INTERNATIONAL ABSTRACTS OF BIOLOGICAL SCIENCES. The Honorary Editor of British Abstracts of Medical Sciences, Dr. C. C. N. Vass, has issued the following statement relative to a change of name and function of this journal.

"The Council of Biological and Medical Abstracts Ltd. has decided to change the title of their journal British Abstracts of Medical Sciences to INTERNATIONAL ABSTRACTS OF BIOLOGICAL SCIENCES.

This decision results from two major developments. The first is an extension of coverage to a wider range of biological research subjects published in journals throughout the world, and the second is that the International Abstracts of Biological Sciences, with the co-operation of the specialist editors selected by the Institute of Scientific Information of the Academy of Sciences of the U.S.S.R. will include, as from the January 1957 issue, translations of the important Russian papers abstracted in the Referativny Zhurnal Biologii (Soviet Biological Abstracts) and Referativny Zhurnal Biologischekoi Khimii (Soviet Abstracts of Biological Chemistry), to be published simultaneously with their appearance in Russian.

International Abstracts of Biological Sciences abstracts the world literature in the following fields: Anatomy, Animal Behaviour, Biochemistry, Biophysics, Cytology, Embryology, Endocrinology, Epidemiology, Experimental Biology, Genetics, Haematology, Histochemistry, Histology, Immunology, Microbiology, Nutrition, Odontology, Parasitology, Pathology, Pharmacology, Physical Anthropology, Physiology, Radiation Effects, Toxicology and Viruses.

International Abstracts of Biological Sciences is published for the Council of Biological and Medical Abstracts Ltd. by Pergamon Press, London and New York, on a nonprofit-making basis."

COMMITTEE ON TAXONOMY OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. President Charles A. Stuart of the Society of American Bacteriologists, acting in pursuance of a resolution of the Council of the Society, has appointed the following to membership on the newly created Committee on Taxonomy.

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY

Dr. Kenneth B. Raper, Chairman, Department of Bacteriology, University of Wisconsin, Madison, Wisconsin.

Dr. Charles A. Evans, Department of Bacteriology, University of Washington, Seattle 5, Washington.

Dr. R. L. Starkey, Department of Microbiology, College of Agriculture, Rutgers University, New Brunswick, New Jersey.

Dr. C. F. Niven, Jr., Department of Microbiology, University of Chicago, Chicago, Illinois.

Dr. R. E. Buchanan, 316 Curtiss Hall, Iowa State College, Ames, Iowa.

Included in the action of the Council was authorization for the appointment of subcommittees interested in the taxonomy and nomenclature of special groups of the bacteria. The first of these subcommittees appointed is the Subcommittee on the Taxonomy of the Actinomycetes, with the following membership:

Dr. David Gottlieb, Chairman, Professor of Plant Pathology, University of Illinois, Urbana, Illinois.

Dr. Robert L. Pittinger, Microbiologist, Eli Lilly and Company, Indianapolis 6, Indiana.

Dr. R. G. Benedict, Microbiologist, Northern Regional Research Branch, Peoria, Illinois.

Dr. K. L. Jones, Professor of Botany, University of Michigan, Ann Arbor, Michigan.

Dr. Ruth Gordon, Microbiologist, Microbiological Institute, Rutgers University, New Brunswick, New Jersey.

DIRECTORY OF SPECIALISTS IN PLANT TAXONOMY. Dr. Reed C. Rollins[1] in a recent article states that the National Science Foundation of the United States has made available a fund which will enable the International Association for Plant Taxonomy to undertake the compilation and publication of a Directory of Specialists in Plant Taxonomy. Included in the outline of the project there is the following statement that is of interest to bacteriologists.

"Certain groups such as bacteria, motile algae, viruses, plant microfossils, etc., sometimes considered to be on the fringe of the plant kingdom are to be included."

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[1]Rollins, Reed C. A directory of specialists. Taxon 5 (6): 117-118. 1956.

The preparation of the Directory will be under the direction of Mr. C. A. de Roon, and under the general supervision of Dr. F. A. Stafleu.

At the request of Dr. Stafleu, the Chairman of the Judicial Commission is forwarding to him the names of bacteriologists who are particularly interested in the taxonomy and nomenclature of the bacteria and viruses. Included in the lists submitted have been the names of the members of the International Committee on Bacteriological Nomenclature and of the several subcommittees.

INDEX OF NAMES OF PLANT GENERA. The International Bureau for Plant Taxonomy and Nomenclature has authorized the organization and publication of an Index Nominum Genericorum (Plantarum) under the direction of Dr. F. A. Stafleu, Editor of TAXON. Specialists in botanical and bacteriological nomenclature have been invited to submit data relative to all validly published names of plant and bacterial genera. These names will be printed on cards giving all pertinent facts, including the name of the genus, name(s) of author(s), citation of place of original publication, a notation if the name is conserved, a notation if the name is rejected (placed in list of nomina rejicienda), the name of the type species (lectotype, neotype), and various notations as to homonymy, nomenclatural synonyms, orthographic variants and the date of publication.

The validly published names of genera of the bacteria are to be included. This inclusion is advantageous both to bacteriologists and botanists. The International Code of Nomenclature of the Bacteria and Viruses provides explicitly that the name of a genus of bacteria is illegitimate if it is a later homonym of a validly published name of a plant genus. An author of a new name of a bacterial genus will for the first time have at hand a check list of the names that are not to be duplicated. Similarly it will enable plant taxonomists to avoid introducing as names of plant genera homonyms of earlier published names of bacterial genera.